COMMUNICATION

Isogalactofagomine lactam. A neutral nanomolar galactosidase inhibitor

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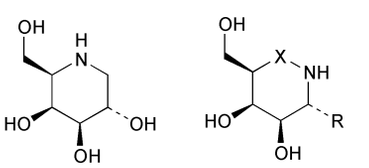
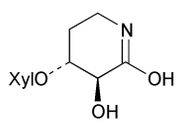
Received (in Cambridge, UK) 27th April 2001, Accepted 6th June 2001
First published as an Advance Article on the web 18th June 2001

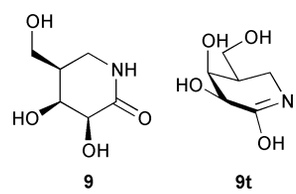
4-Deoxy-4-hydroxymethyl-L-ribono-1,5-lactam (9) was synthesised and found to be a potent inhibitor of β-galactosidase from *Aspergillus oryzae* with a K_i of 18 nM.

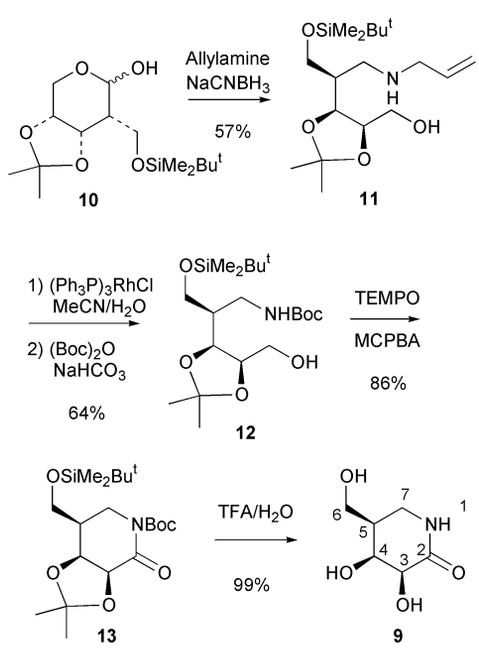
Most potent glycosidase inhibitors are basic aminosugars,[1] such as **1**,[2] **2**,[3] **3**[4] or **4**,[5] and are able to become positively charged. (For some new applications of glycosidase inhibitors, see ref. 6.) Nevertheless neutral glyconolactams, such as **5**,[2,7] have long been known to inhibit glycosidases.[8] The enzyme inhibition by these lactams, and the corresponding lactones, was widely interpreted as evidence for a flat transition state and has had a heavy impact on the common perception of the transition state of glycoside cleavage. However recent research suggests that the glycosidase inhibition by **5** and similar compounds may not be due to the flat structure but rather to H-bonding of the carbonyl group.[9] Recently a new type of glycosidase inhibiting lactam was reported.[10,11] Withers has reported that the lactam **6**, an analogue of xylobiose-type 1-azasugar **7**, is almost as potent (K_i 340 nM) as **7** (K_i 130 nM) against a β-xylosidase. This is remarkable since the strong binding of **7** is believed to be associated with salt bridge formation from N.[10] The affinity of **6** was explained with the tautomeric form of the amide **6t** being able to act both as an amine and as a 2-hydroxy group. Vasella has reported that the hydrazinolactam **8** is a much stronger α-mannosidase inhibitor than the *gluco*-isomer of **4** (**8**: K_i 25 μM, *gluco*-**4**: K_i 3.3 mM) albeit a weaker β-glucosidase inhibitor (**8**: K_i 13 μM, *gluco*-**4**: K_i 0.32 μM).[11] We have decided to further explore the generality of this idea, and report the synthesis and biological activity of galactose-type lactam **9**.

Synthesis of **9** started from optically active 2-deoxy-2-hydroxymethyl-D-ribose derivative **10**, available from D-arabinose in 6 steps.[12] Reductive amination of **10** with CH_2=$CHCH_2NH_2$ and $NaCNBH_3$ in MeOH–H_2O (pH 6, adjusted with AcOH) gave the amine **11** in 57% yield (Scheme 1). The allyl group was removed with Wilkinson's catalyst









Scheme 1

(1 equiv.) in MeCN–H_2O (6 : 1) at reflux (3 h). The resulting amine was immediately Boc-protected (Boc_2O, $NaHCO_3$) to give the Boc-protected amine **12** in 64% yield from **11**. Oxidation of **12** using 2,2,6,6-tetramethylpiperidine *N*-oxide (TEMPO, 0.7 equiv.) and *m*-chloroperbenzoic acid (MCPBA, 30 equiv.) as cooxidant with Bu_4NBr in CH_2Cl_2 gave the lactam **13** in 86% yield. Deprotection of **13** was carried out by treatment with trifluoroacetic acid–H_2O (5 : 1, 5 min, 25 °C) to give **9** in essentially quantitative yield.[13]

 DOI: 10.1039/b103831c

Table 1 Inhibition (dissociation) constants K_i/μM of **1**–**5** and **9** for various glycosidases. Measured at pH 6.8 and 25 °C

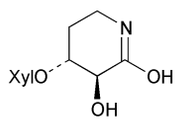
Enzyme	**1**	**2**	**3**	**4**	**5**	**9**
α-Galactosidase (coffee bean)	0.002	50	0.7	0.28	>1000[a]	5.2
β-Galactosidase (*A. oryzae*)	—	0.004	0.035	0.04	4.5[a]	0.018
β-Galactosidase (*S. fragilis*)	81	0.33	0.09	7.8	13.0[b]	9.0
β-Glucosidase (almond)	540	0.08	0.3	0.13	85	3.0

[a] Enzyme from *Aspergillus niger*. [b] Enzyme from *Penicillium multicolor*.

Lactam **9** was tested for inhibition of three galactosidases and β-glucosidase. For all four enzymes competitive inhibition was observed, and the K_i values at pH 6.8 and 25 °C were determined and compared with those of inhibitors **1**–**5** (Table 1). Compound **9** was found to be a remarkably potent inhibitor of β-galactosidase from *A. oryzae* with a K_i of 18 nM. Against this enzyme it is only surpassed in potency by the isofagomine **2** (4 fold weaker), but **9** is two fold more potent than the strong inhibitors **3** and **4**. The lactam **9** is much less potent against the three other enzymes, but it is still a rather good inhibitor with K_i in the low micromolar range. It is stronger than **1** against β-galactosidase and stronger than **2** against α-galactosidase and generally, from the data available, appears more potent than the lactam **5**.

It is particularly remarkable that **9** is so potent against β-galactosidase from *A. oryzae*, because this enzyme appears to be the least sensitive to the presence of a 2-hydroxy group in the inhibitor. In fact the noeuromycin **3**, which has a 2-hydroxy group is weaker than its 2-deoxy analogue **2** and also weaker than **9**. It therefore appears not to make sense that the lactam carbonyl group should mimic a hydroxy group in this case. In contrast β-galactosidase from *S. fragilis*, which in fact appears sensitive to the presence of a 2-hydroxy group, since **3** is more potent than **2** against this enzyme, is relatively weakly inhibited by **9**. However, these observations may be explained by **9**, in its half-chair geometry, having the 2-carbonyl group better positioned for H-bonding to some enzymes than for **3** and *vice versa*, depending on the geometry of the transition state in a particular enzyme. It is unlikely that the tautomer **9t** is the binding species with the *Aspergillus oryzae* enzyme, because **3** would be expected to be a more potent inhibitor than **9**, since it is less costly to twist **3** from a chair into a half-chair conformation (~ 20 kJ mol^{-1}) than to convert an amide into an iminol (~45 kJ mol^{-1}). It should also be noted that while the half-chair geometry of these lactams may cause the carbonyl group to be well positioned for hydrogen bonding, the geometry does not appear to improve the interactions of the nitrogen atom, since the hydrazone **14** is a very poor glycosidase inhibitor[14] even though it has a geometry that is almost superimposable with that of **8**.

In the present work we have shown that lactam **9** is a potent galactosidase inhibitor. The potency of its inhibition is, however, highly dependent on the source of the enzyme. It is likely that similar 2-hydroxymethylpentonolactams that mimic other



6t

monosaccharides may be potent inhibitors of the corresponding glycosidases.

Acknowledgements

We acknowledge financial support from the Danish National Science Research Council (THOR program) and from the Lundbeck foundation.

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13 All new compounds gave satisfactory HRMS, ^{1}H and ^{13}C NMR spectra. NMR data for **9**: ^{1}H NMR (200 MHz, D_2O) δ 4.07 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 2.0 Hz, H-4), 4.02 (d, 1H, H-3), 3.53 (dd, 1H, $J_{5,6a}$ 7.9 Hz, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.40 (dd, 1H, $J_{5,6b}$ 6.8 Hz, H-6b), 3.15 (dd, 1H, $J_{5,7eq}$ 6.0 Hz, $J_{7ax,7eq}$ 12.4 Hz, H-7eq), 2.92 (t, 1H, $J_{5,7ax}$ 12.4 Hz, H-7ax), 2.20–2.10 (m, 1H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.1 (C-2), 70.4, 67.9 (C-3, C-4), 61.2 (C-6), 40.4, 38.7 (C-5, C-7).
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